Lack of Correlation between the Response to a Proliferation Inhibitor and Other Transformation Markers in a Mutant Liver Cell Line

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ABSTRACT

A rat liver factor, which has been found previously to inhibit proliferation of untransformed rat liver cell lines but not of transformed liver cell lines, did not inhibit proliferation of the chemically transformed rat liver cell line, W-8. Moreover, a temperature-sensitive mutant derived from W-8 (TS-223), which exhibits an untransformed phenotype at 39.5-41°C and a transformed phenotype at 36°C, was not affected by the liver factor at either temperature. Since the factor can be incubated at 41°C for 4 days without loss of activity, it would seem that the regulation of cell proliferation is not necessarily linked with the expression of other markers of transformed cells.

INTRODUCTION

We reported recently the partial purification of a factor from adult rat liver which inhibits the proliferation of nonmalignant rat liver cells (8). When the effect of this factor on the proliferation of a number of untransformed rat liver cell lines and transformed or malignant hepatoma cell lines was studied, it was found that division of all untransformed cells was inhibited by the hepatic factor and that the inhibition was reversed on removal of the factor. In contrast, the division of the transformed liver cells was either not inhibited or slightly stimulated. To see whether this altered response to proliferation control in the transformed liver cells is associated, or linked, with other markers of transformed cells, we studied the well-characterized temperature-sensitive mutant rat liver cell line, TS-223, and its parental transformed cell line, W-8 (14, 15). At the permissive temperature, the TS-223 cells exhibited growth characteristics and transformation markers similar to those of the wild type W-8 cells. Even at the nonpermissive temperatures (39.5-41°C), the W-8 cells exhibited all the characteristics expressed at 36°C, but the TS-223 cells showed a reduction in saturation density and cloning efficiency in liquid medium (14, 15). Moreover, when maintained at 39.5-41°C, their plating efficiency in semisolid agar was negligible, and they attained a cell morphology and cell surface architecture (as studied by scanning electron microscopy) comparable with that of the untransformed parental cells (15). The separation profile of fucose-labeled glycopeptides isolated by trypsinization (10) from TS-223 cells maintained at 40°C was also similar to that isolated from the nonmalignant liver cells. All these properties reverted back when the temperature was shifted to 36°C. We report here that the proliferation of TS-223 cells was not inhibited by the hepatic proliferation inhibitor either at the permissive temperature, where other transformation markers are expressed, or at the nonpermissive temperature, where other markers are suppressed.

RESULTS

The effect of the partially purified hepatic factor (8) on the proliferation of rat liver epithelial cell lines is shown in Table 1. This material significantly inhibited \( p < 0.001 \) the proliferation of the untransformed liver cell line FNRL-1 at 36°C as evidenced by the reduced number of large cell colonies (Experiment 1). Moreover, the fraction of smaller colonies in the total population was concomitantly increased. Test media preincubated at 41°C

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\[ ^{3} \text{The abbreviation used is: DMEM, Dulbecco's modified Eagle's medium.} \]
for 4 days also showed a similar level of inhibition of the large colonies in this cell line (Experiment 2). The liver factor did not inhibit the proliferation of the transformed W-8 cells at either 36° or 41° (Experiment 3). This cell line also had a higher plating efficiency at 41°. In contrast, the temperature-sensitive mutant cell line, TS-223, did not form colonies when cultured at 41° (Experiment 4) in DMEM. Two conditions (Experiments 5 and 6) which allowed the TS-223 cell line to form colonies at higher temperatures were therefore used. When the temperature was kept at 39.5° in DMEM or when maintained at 41° in Ham’s F-10 medium, the plating efficiencies at the nonpermissive temperatures were 18.5 and 4.1, respectively. The proliferation of the TS-223 cell line was not inhibited by the liver factor at the permissive temperature (36°) or at the nonpermissive temperatures (39.5° or 41°) in these 2 experiments.

**DISCUSSION**

Since loss of control of cell proliferation is an important, and possibly irreversible, key alteration in carcinogenesis, we attempted to isolate endogenous regulators of cell proliferation. Recently, we reported the partial purification of a factor from rat liver which, while inhibiting cell division in a variety of untransformed rat liver epithelial cell lines, had either no effect or had a growth-promoting activity on malignant liver cells transformed in vitro or in vivo (8). The availability of temperature-sensitive mutants of chemically transformed liver cells (14, 15) which exhibit transformation markers predominantly at the permissive (36°) temperature (10, 14, 15) allowed us to determine whether the liver factor would exert a preferential inhibition of cell proliferation of this cell line at nonpermissive temperatures, where the phenotype is untransformed.

This could be examined, because cells blocked in cell cycle by the hepatic factor (4 days) and maintained thereafter in regular culture medium for an additional 5 days should yield more smaller colonies and fewer larger colonies than untreated control cell populations. The inhibition by the factor is shown by the reduction of the number of larger colonies formed compared with the control, and the predominantly cytostatic nature of this inhibition is indicated by the increase in the fraction of smaller colonies. Using these criteria, we have shown that the hepatic factor inhibits the untransformed liver cell line, FNRL-1 (Experiment 1). Cytostatic inhibition by endogenous factors has been implicated in the regulation of cell proliferation (2). However, such factors isolated from liver inhibited the incorporation of thymidine by malignant liver cells (inter alia Refs. 1, 7, 11, and 12). In this report, we have taken advantage of the specificity of the hepatic proliferation inhibitor towards the division of nonmalignant liver cells to study whether the expression of this marker was linked to other markers of transformed liver cells.

**Table 1**

**Effect of a partially purified hepatic factor on the proliferation of rat liver cell lines**

<table>
<thead>
<tr>
<th>Target liver cells</th>
<th>Experiment</th>
<th>Culture medium</th>
<th>Temperature</th>
<th>No. of cells plated</th>
<th>[Hepatic factor] (μg/ml)</th>
<th>PE</th>
<th>Smaller colonies (&lt;0.5 mm)</th>
<th>No. of large colonies (&gt;0.5 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FNRL-1 (untransformed)</td>
<td>1</td>
<td>F-10</td>
<td>36°</td>
<td>1 x 10⁵</td>
<td>0</td>
<td>12.2</td>
<td>0.48</td>
<td>63 ± 15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F-10</td>
<td>36°</td>
<td>1 x 10⁵</td>
<td>0</td>
<td>5.8</td>
<td>0.76</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>W-8 (transformed; wild type)</td>
<td>3</td>
<td>DMEM</td>
<td>36°</td>
<td>1 x 10⁵</td>
<td>0</td>
<td>16.7</td>
<td>0.44</td>
<td>94 ± 15</td>
</tr>
<tr>
<td>TS-223 (transformed; temperature-sensitive)</td>
<td>4</td>
<td>DMEM</td>
<td>36°</td>
<td>3 x 10²</td>
<td>0</td>
<td>20.3</td>
<td>0.51</td>
<td>30 ± 3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>DMEM</td>
<td>36°</td>
<td>5 x 10²</td>
<td>0</td>
<td>30.4</td>
<td>0.20</td>
<td>122 ± 10</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>F-10</td>
<td>36°</td>
<td>3 x 10²</td>
<td>0</td>
<td>29.3</td>
<td>0.41</td>
<td>52 ± 7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>F-10</td>
<td>36°</td>
<td>1 x 10⁴</td>
<td>0</td>
<td>4.1</td>
<td>0.62</td>
<td>156 ± 39</td>
</tr>
</tbody>
</table>

* PE, total number of colonies/number of cells plated × 100.
* Mean ± S.D. obtained from 6 replicate dishes.
* The F-10 medium (with or without the hepatic factor) was preincubated at 41° for 4 days and then tested.
and to see whether the loss of response towards the hepatic study using nonmalignant rat liver cells undergoing transformation is required to test the generality of these observations and to see whether the loss of response towards the hepatic proliferation inhibitor is an earlier event compared with other markers of transformation.

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